

Fig. 1

1B

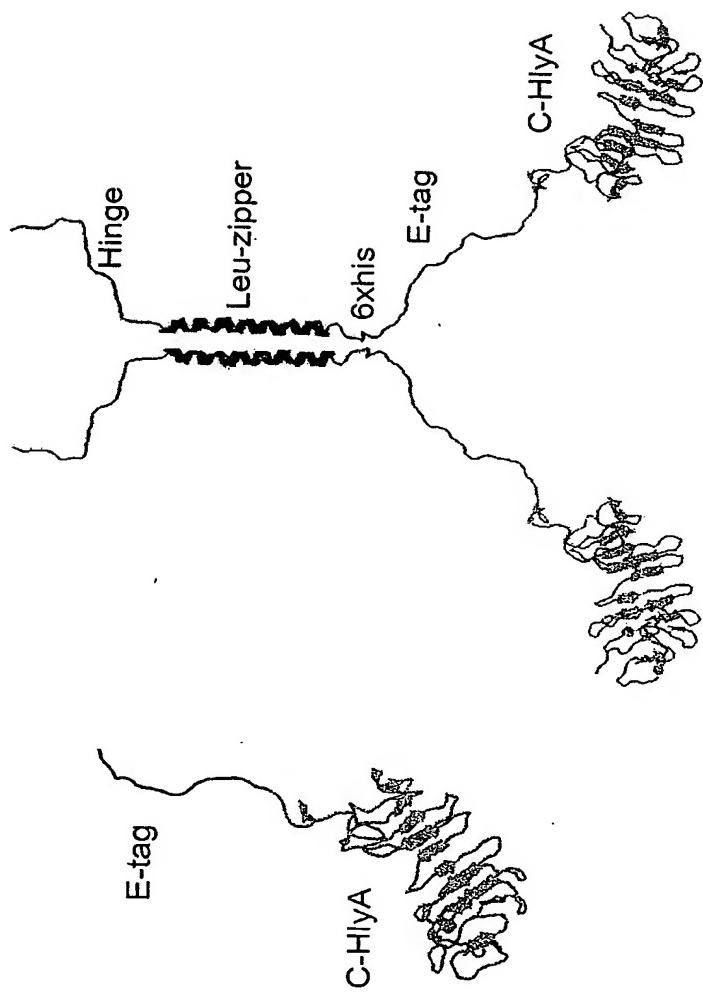


Fig. 1 (cont.)

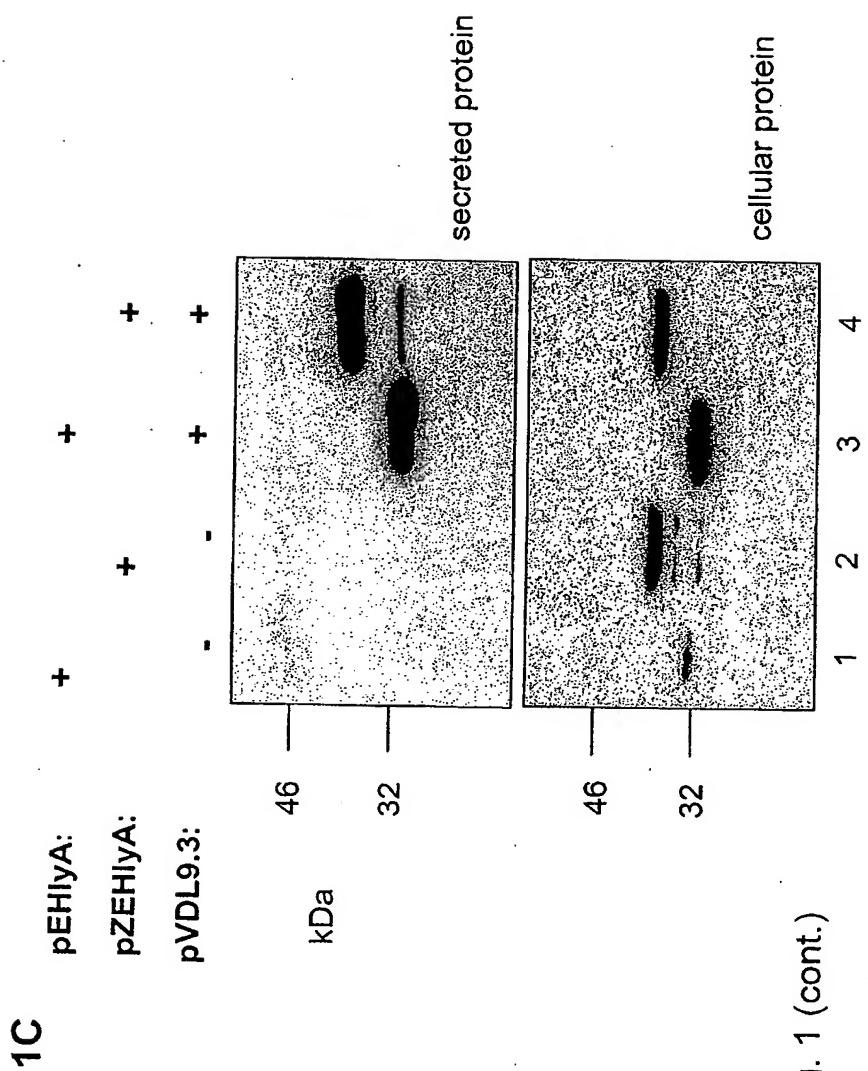


Fig. 1 (cont.)

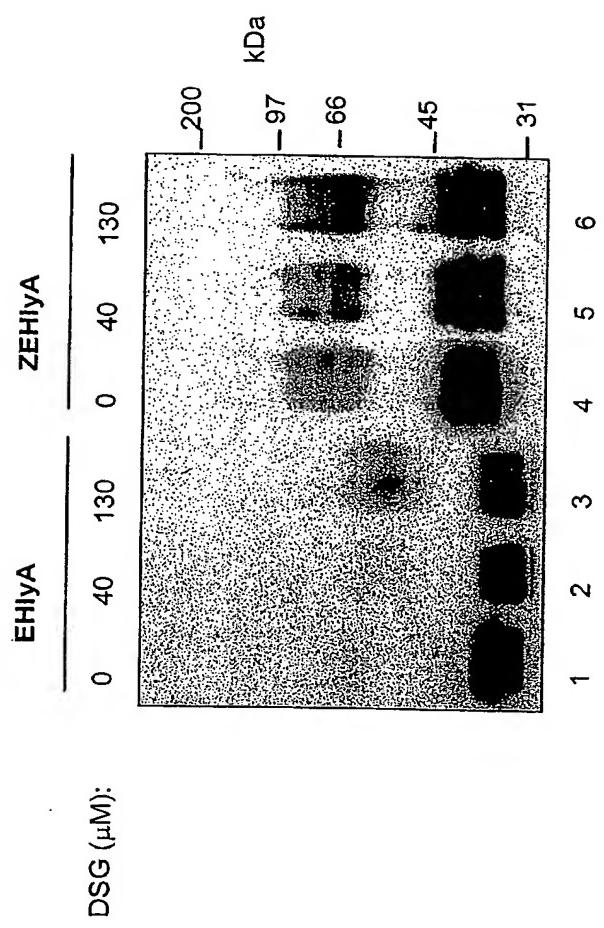


Fig. 2

3A

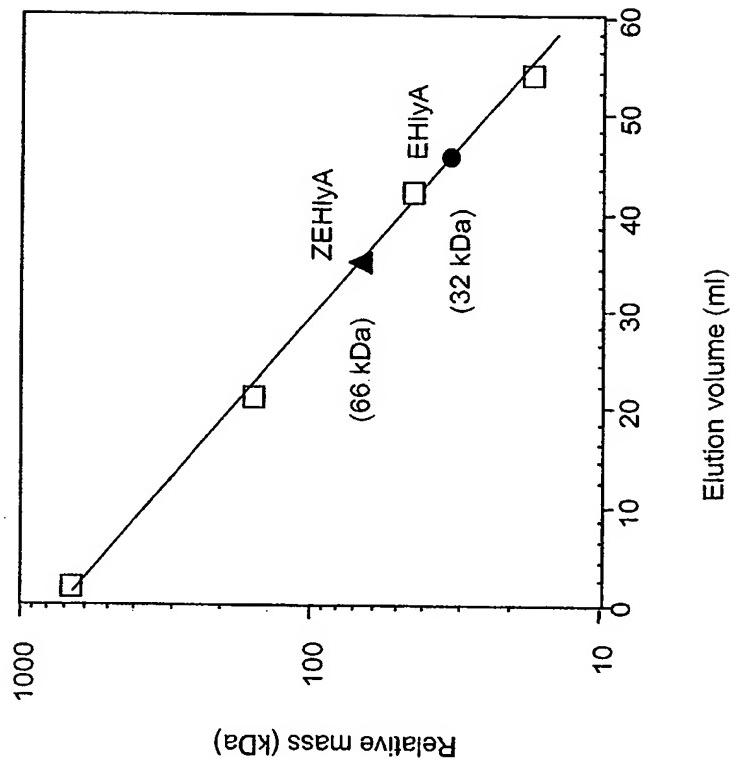
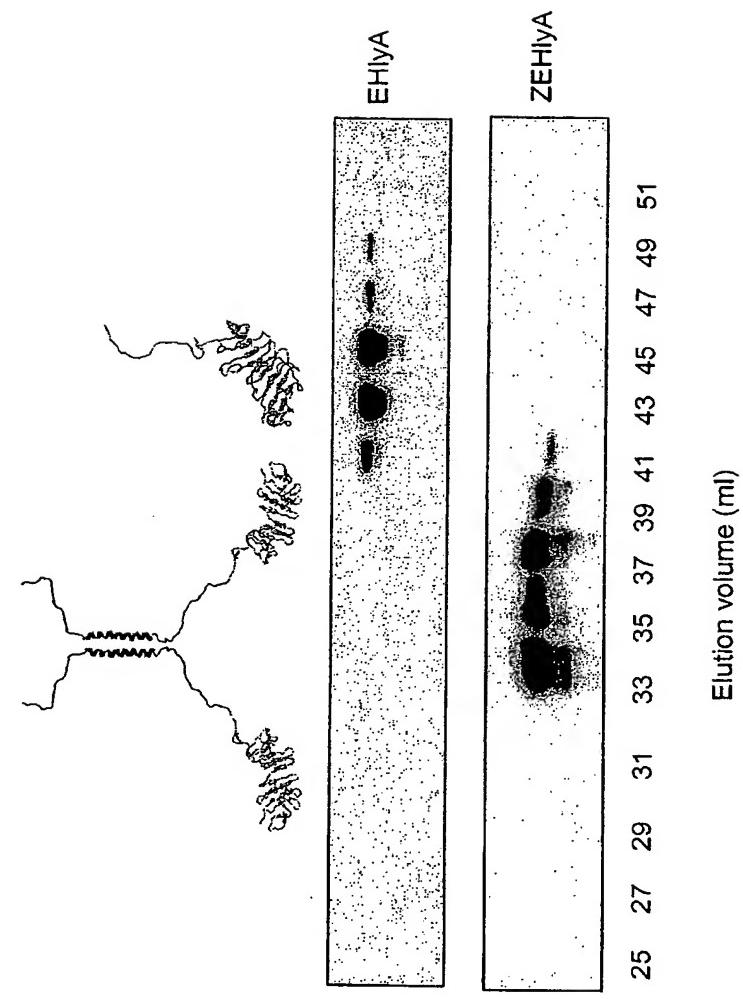


Fig. 3

3B

Fig. 3 (cont.)



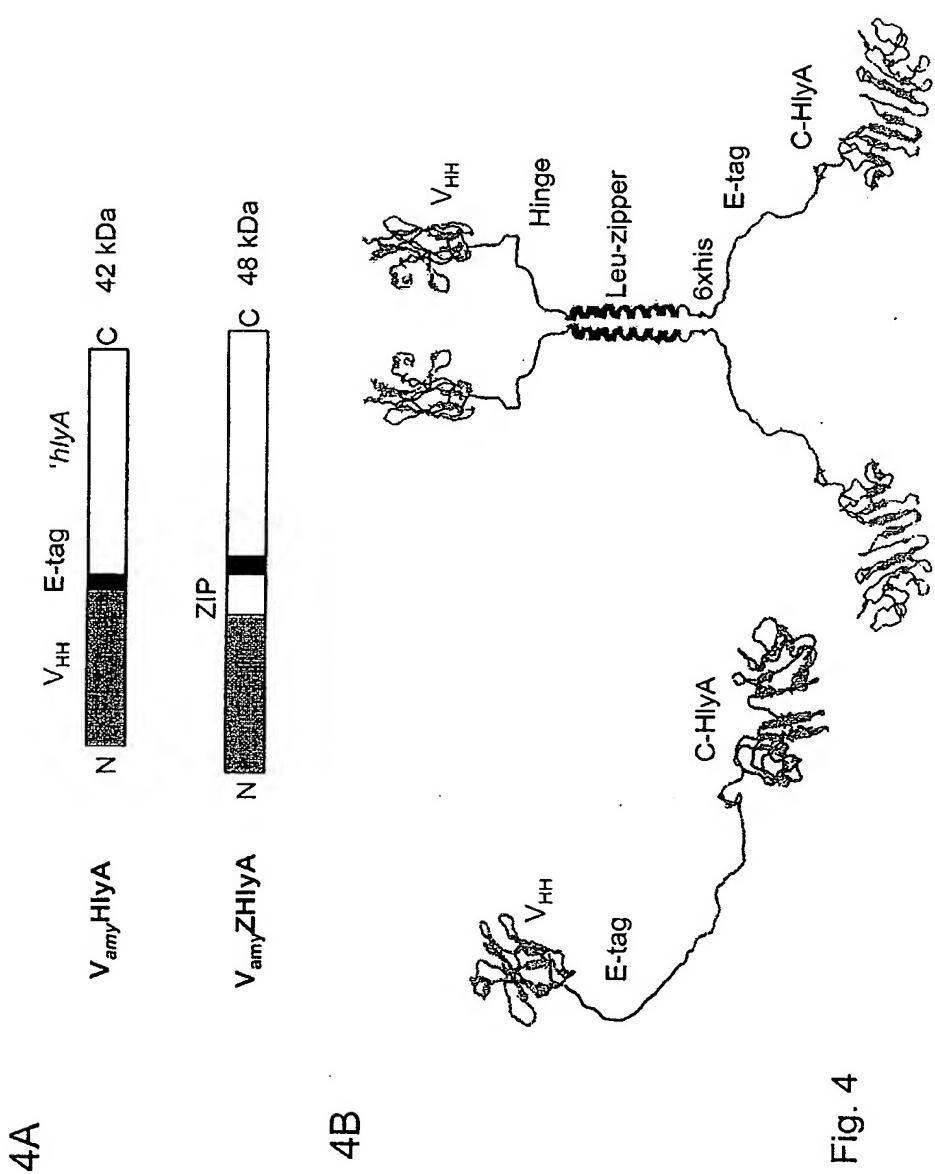


Fig. 4

4C
4D

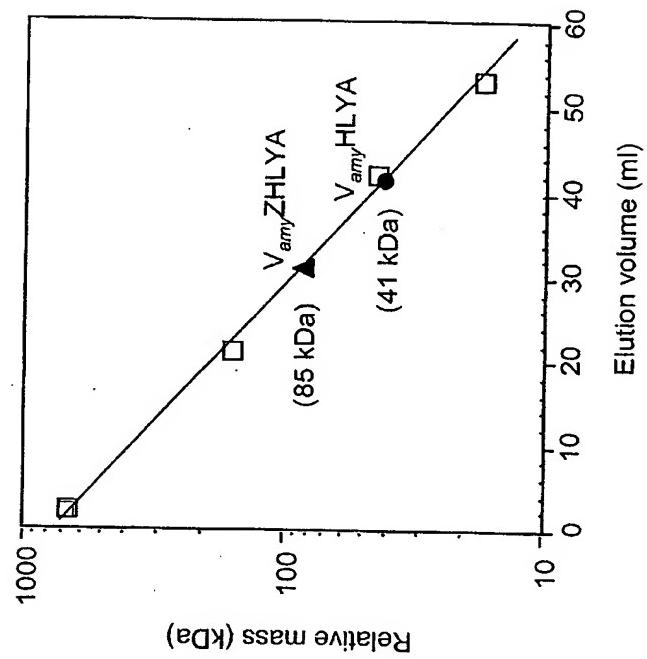
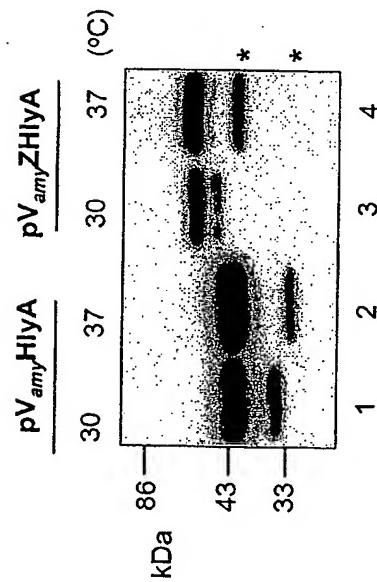
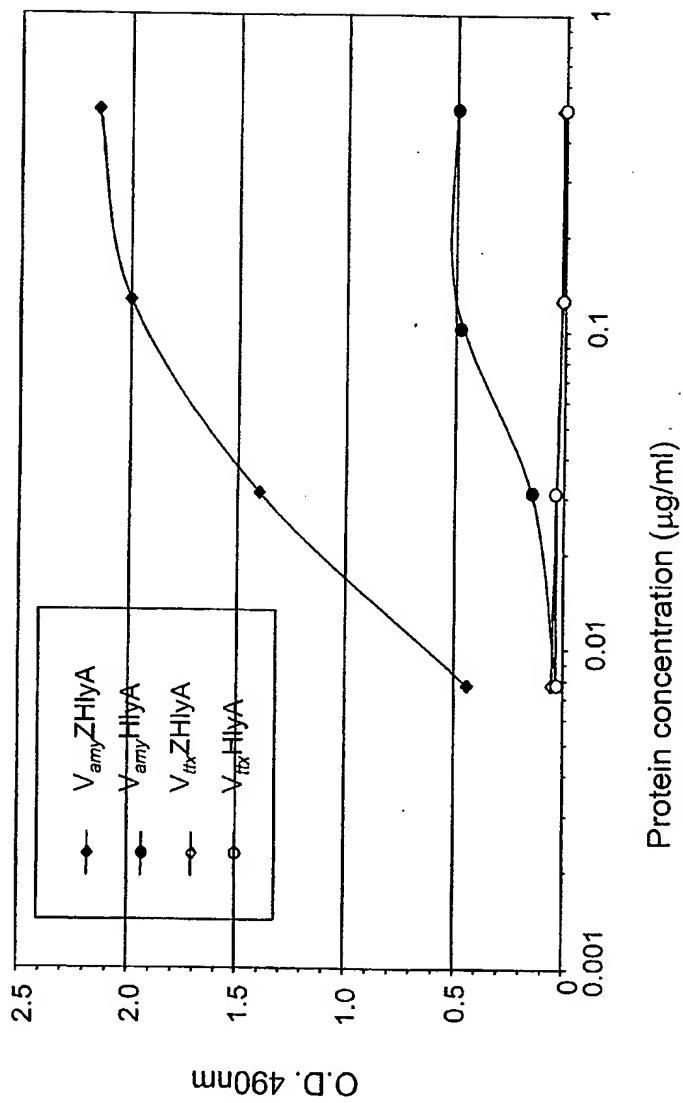


Fig. 4 (cont.)

Fig. 5



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p2EhlyA map

With 16 restriction enzymes: ECORI PSTI SALI HINDIII BAMHI NOTI SACI
SACII SPHI KPNI XBAI NHEI NDEI

AGCGGATAACAATTCACACAGGAAACAGCT (sequence before the ATG)

Fig. 6

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GGTAATGACAAGTTGTACGGCAGTGAGGGAGCAGACCTGTTGATGGCGGAGAAGGAAAT
301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 360
CCATTACTGTTAACATGCCGTCACTCCCTCGTCTGGACGAACTACCGCCTCTTCCCTTA

a G N D K L Y G S E G A D L L D G G E G N -

GATCTTCTGAAAGGTGGATATGGTAATGATATTTATCGTTATCTTCAGGATATGGCAT
361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 420
CTAGAAGACTTTCCACCTATAACCATTACTATAAAATGCAATAGAAAGTCCTATACCGGTA

a D L L K G G Y G N D I Y R Y L S G Y G H -

CATATTATTGACGATGAAGGGGGAAAGACGATAAACTCAGTTAGCTGATATAGATTTC
421 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
GTATAATAACTGCTACTTCCCCCTTCTGCTATTTGAGTCAAATCGACTATATCTAAAG

a H I I D D E G G K D D K L S L A D I D F -

CGGGACGTTGCCTTAAGCGAGAAGGAAATGACCTCATTATGTATAAAGCTGAAGGTAAT
481 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
GCCCTGCAACGGAAATTCGCTTCCCTACTGGAGTAATAACATATTCGACTTCCATTA

a R D V A F K R E G N D L I M Y K A E G N -

GTTCTTCTATTGCCACAAAATGGTATTACATTAAAAACTGGTTGAAAAAGAGTC
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
CAAGAAAAGATAACCGGTGTTTACCATATAATGAAATTGGACCAAACCTTTCTCAGT

a V L S I G H K N G I T F K N W F E K E S -

GATGATCTCTAAATCATCAGATAGAGCAGATTGGATAAAAGACGGCAGGGTAATCACA
601 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 660
CTACTAGAGAGATTAGTAGTCTATCTCGTCTAAACTATTCTGCCGTCCCATTAGTGT

a D D L S N H Q I E Q I F D K D G R V I T -

CCAGATTCTCTAAAAAGCATTGAAATATCAGCAGAGTAATAACAAGGTAAGTTATGTG
661 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
GGTCTAAGAGAATTTCGTAACCTATAGTCGTCATTATTGTTCCATTCAACAC

a P D S L K K A F E Y Q Q S N N K V S Y V -

TATGGACATGATGCATCAACTTATGGGAGCCAGGACAATCTTAATCCATTAAATGAA
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 780
ATACCTGTACTACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGTAATTAAATTACTT

a Y G H D A S T Y G S Q D N L N P L I N E -

Fig. 6 (cont.)

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PstI

|

ATCAGCAAAATCATTCTAGCTGCAGGTAACCTCGATGTTAAGGAGGAAAGATCTGCCGCT
781 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 840

TAGTCGTTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCCTTCTAGACGGCGA

a I S K I I S A A G N F D V K E E R S A A -

NdeI

|

TCTTTATTGCAGTTGTCGGTAATGCCAGTGATTTTCATATGGACGGAACCTCAATAACT
841 -----+-----+-----+-----+-----+-----+-----+-----+ 900

AGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTATACTGCCTTGAGTTATTGA

a S L L Q L S G N A S D F S Y G R N S I T -

TTGACAGCATCAGCATAA
901 -----+----- 918

AACTGTCGTAGTCGTATT

a .L T A S A * -

Cutting enzymes:

AccI	BamHI	EcoRI	HindIII	NdeI	PstI	SacI	SalI
SmaI	XbaI						

Not cutting enzymes:

KpnI	NcoI	NheI	NotI	SacII	SphI		
------	------	------	------	-------	------	--	--

Fig. 6 (cont.)

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pZEHLYA2SD map

With 11 restriction enzymes: ECORI BGLII BAMHI NCOI NHEI SFI I SALI XMAI
XBAI EAGI SALI HINDIII

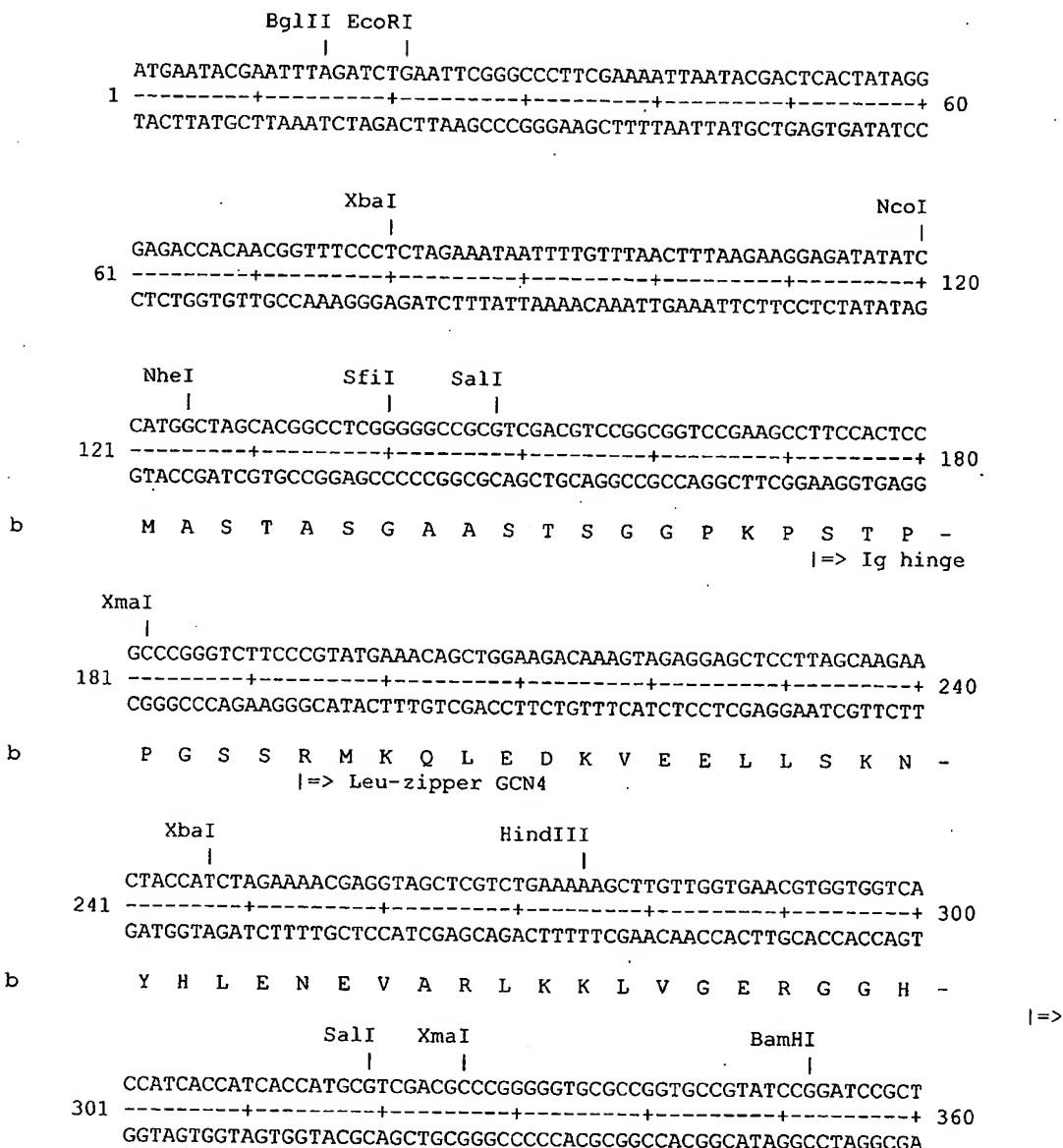


Fig. 7

b H H H H H A S T P G G A P V P Y P D P L -
 6xhis tag |=> E-tag
 EagI
 |
 361 GGAACCGGCCGGGAAAATTCTCTTGCATAAAATGTATTATCCGGTGGAAAAGGTAATGA
 -----+-----+-----+-----+-----+-----+ 420
 CCTTGGCCGGCCCCTTTAAGAGAACGATTTCACATAATAGGCCACCTTCCATTACT

b E P A G E N S L A K N V L S G G K G N D -
 |=> C-hlyA
 421 CAAAGTTGTACGGCAGTGAGGGAGCAGACCTGCTTGTGATGGCGGAGAAGGGAATGATCTTCT
 -----+-----+-----+-----+-----+-----+ 480
 GTTCAACATGCCGTCACTCCCTCGTCTGGACGAACCTACCGCCTTCCCTACTAGAAGA

b K L Y G S E G A D L L D G G E G N D L L -
 481 GAAAGGTGGATATGGTAATGATATTATCGTTATCTTCAGGATATGCCCATCATATTAT
 -----+-----+-----+-----+-----+-----+ 540
 CTTTCACCTATACCACTACTATAAATAGCAATAGAAAGTCCTATACCGTAGTATAATA

b K G G Y G N D I Y R Y L S G Y G H H I I -
 541 TGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCTGATATAGATTCCGGGACGT
 -----+-----+-----+-----+-----+-----+ 600
 ACTGCTACTTCCCCCTTCTGCTATTGAGTCAAATCGACTATATCTAAAGGCCGTGCA

b D D E G G K D D K L S L A D I D F R D V -
 601 TGCCTTAAGCGAGAAGGGAATGACCTCATTATGTATAAGCTGAAGGTAATGTTCTTTC
 -----+-----+-----+-----+-----+-----+ 660
 ACGGAAATTGCTCTCCCTACTGGAGTAATACATATTCGACTTCATTACAAGAAAG

b A F K R E G N D L I M Y K A E G N V L S -
 661 TATTGGCCACAAAATGGTATTACATTAAAACTGGTTGAAAAGAGTCAGATGATCT
 -----+-----+-----+-----+-----+-----+ 720
 ATAACCGGTGTTTACCATATGTAAATTGACCAAACCTTCTCAGTCTACTAGA

b I G H K N G I T F K N W F E K E S D D L -
 721 CTCTAACATCAGATAGAGCAGATTTGATAAAAGACGGCAGGGTAATCACACCAGATT
 -----+-----+-----+-----+-----+-----+ 780
 GAGATTAGTAGTCTATCTCGTCTAAAACATTCTGCCGTCCCATTAGTGTGGTCTAAG

b S N H Q I E Q I F D K D G R V I T P D S -
 781 TCTAAAAAAAGCATTTGAATATCAGCAGAGTAATAACAAGGTAAGTTATGTGTATGGACA
 -----+-----+-----+-----+-----+-----+ 840
 AGAATTTCGTAACCTATAGTCGTCATTATTGTTCCATTCAATACACATACTGT

Fig. 7 (cont.)

b L K K A F E Y Q Q S N N K V S Y V Y G H -
 TGATGCATCAACTTATGGGAGGCCAGGACAATCTTAATCCATTAAATTAATGAAATCAGCAA
 841 -----+-----+-----+-----+-----+-----+-----+ 900
 ACTACGTAGTTGAATAACCCTCGGTCTGTTAGAATTAGGTAATTAATTACTTAGTCGTT

b D A S T Y G S Q D N L N P L I N E I S K -
 AATCATTTAGCTGCAGGTAACCTCGATGTTAAGGAGGAAAGATCTGCCGCTTCTTTATT
 901 -----+-----+-----+-----+-----+-----+-----+ 960
 TTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCCTTCTAGACGGCGAAGAAATAA

b I I S A A G N F D V K E E R S A A S L L -
 GCAGTTGTCGGTAATGCCAGTGATTTTCAATGGACGGAACCTAATAACTTGACAGC
 961 -----+-----+-----+-----+-----+-----+-----+ 1020
 CGTCAACAGGCCATTACGGTCACTAAAAAGTATACCTGCCCTGAGTTATTGAAACTGTGCG

b Q L S G N A S D F S Y G R N S I T L T A -
 ATCACGCATAATATATTAATTAAATGATAGCAATCTTACTGGGCTGTGCCACATAAGATT
 1021 -----+-----+-----+-----+-----+-----+-----+ 1080
 TAGTCGTATTATATAATTAAATTTACTATCGTTAGAATGACCCGACACGGTGTATTCTAA

b S A * -
 GCTATTTTTGGAGTCATAATGGATTCTTGTCAAAAATTGATTATGGGTATACGCC
 1081 -----+-----+-----+-----+-----+-----+-----+ 1140
 CGATAAAAAAACCTCAGTATTACCTAAGAACAGTATTAACTAATACCCAATATGCGGG

TGGAGATTTAGCCAATACCATAACGTCTGTAAACCCGGAAGAAATTAAACATAGAT
 1141 -----+-----+-----+-----+-----+-----+-----+ 1200
 ACCTCTAAAATCGGGTTATGGTATTGCAGAGACAATTGGCCTCTTTAATTGTATCTA

TTGACACAGACGGGACTGGCTGGGATTAACGTCACTGGTCTGCTGCGAAATCTTAG
 1201 -----+-----+-----+-----+-----+-----+-----+ 1260
 AACTGTGTCTGCCCTGACCAGACCCCTATTGCACTACCAACGAACGACGCTTAGAAATC

AACTAAAGGTAAAACAGGTAAAAAAACAATTGACCGATTAACTTATTCTCTGCCCG
 1261 -----+-----+-----+-----+-----+-----+-----+ 1320
 TTGATTTCCATTTGTCCTTTTGTAACTGGCTAATTGAAATAAGAGACGGGC

CATTAGTCTGGAGAGAGGATGGACGTCACTTATTCTGACTAAAGTCAGTAAAGAACAA
 1321 -----+-----+-----+-----+-----+-----+-----+ 1380
 GTAATCAGACCTCTCCTACCTGCAGTAAAATAAGACTGATTTCAGTCATTCTCGTT

Fig. 7 (cont.)

Fig. 7 (cont.)

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Cutting enzymes:

BamHI	BglII	EagI	EcoRI	HindIII	NcoI	NheI	SalI
SfiI	XbaI	XmaI					

Not cutting enzymes:

None

Fig. 7 (cont.)

pVamyHYA map

With 8 enzymes: NCOI PSTI SALI HINDIII SFII BAMHI NOTI ECORI PSTI

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ACTATAGGGAGACCACAAACGGTTCCCTCTAGAAATAATTTGTTAACCTTAAGAAGGA      60
1 -----+-----+-----+-----+-----+-----+
TGATATCCCTCTGGTGTGCCAAAGGGAGATCTTATTAAAACAATTGAAATTCTTCCT

NcoI
|
GATATATCCATGGCTCAGGTGCAGCTGGTGGAGTCTGGGGAGGCTCGGTGCAGGCTGGG      120
61 -----+-----+-----+-----+-----+-----+
CTATATAGGTACCGAGTCCACGTCGACCACCTCAGAACCCCTCCGAGCCACGTCCGACCC

a      M A Q V Q L V E S W G G S V Q A G -
|=> VHH amylase

GGGTCTCTGAGACTCTCCTGCACAGCCCCCTGGATTCACCTCCAATAGCTGCCGCATGGAC      180
121 -----+-----+-----+-----+-----+-----+
CCCAAGAGACTCTGAGAGGACGTGTCGGGACCTAAGTGGAGGTTATCGACGGCGTACCTG

a      G S L R L S C T A P G F T S N S C R M D -
PstI
|
TGGTACCGCCAGGCTGCAGGGAAAGCAGCGCAGTGGGTCTCATCTATTAGTACTGATGGT      240
181 -----+-----+-----+-----+-----+-----+
ACCAAGGCGGTCCGACGTCCCTCGTCGCGCTACCCAGAGTAGATAATCATGACTACCA

a      W Y R Q A A G K Q R E W V S S I S T D G -
CGCACAAAGCTATGCAGACTCCGTGAAGGGCCGATTACCATCTCAAAGACAAAGCCAAG      300
241 -----+-----+-----+-----+-----+-----+
GCGTGTTCGATACGTCTGAGGCACTTCCCGCTAAGTGGTAGAGGTTCTGTTCGGTTCTG

a      R T S Y A D S V K G R F T I S K D K A K -
GACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGCCATCTATTACTGT      360
301 -----+-----+-----+-----+-----+-----+
CTGTGCCACATAGACGTTACTTGTCCGACTTGGACTCCTGTGCCGGTAGATAATGACA

a      D T V Y L Q M N S L K P E D T A I Y Y C -
GCCGTGAGGACGAATGGGTATCGTCCGCAATCTCACGAATTCGCTACTGGGGCCCGGGG      420
361 -----+-----+-----+-----+-----+-----+
CGGCACCTCGCTTACCCATAGCAGGCGTAGAGTGCTTAAAGCGATGACCCCGGGCCCC

a      A V R T N G Y R P Q S H E F R Y W G P G -

```

Fig. 8

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SfiI SalI
| |
421 ACCCAGGTACCGTCTCCCAACGGCCTCGGGGGCCGCGTCGACGCCCGGGGTGCGCCG
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
TGGGTCCAGTGGCAGAGGAGTTGCCGGAGCCCCCGGCAGCTGCGGGCCCCACGCGGC

a T Q V T V S S T A S G A A S T P G G A P -
|=> E-tag
BamHI
|
481 GTGCCGTATCGGATCCGCTGGAACCGGCCGGgAAAAAtCTCTTGCTAAAATGTATTAA
-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
CACGGCATAGGCCTAGGCGACCTTGGCCGGCCcTTTTAaGAGAACGATTTTACATAAT

a V P Y P D P L E P A G E N S L A K N V L -
|=> C-hlyA

TCCGGTGGAAAAGGTAATGACAAGTTGACGGCAGTGAGGGAGCAGACCTGCTGATGGC
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
AGGCCACCTTTCCATTACTGTTCAACATGCCGTCACTCCCTCGTCTGGACGAACCTACCG

a S G G K G N D K L Y G S E G A D L L D G -
GGAGAAGGGAATGATCTTCTGAAAGGTGGATATGGTAATGATATTATCGTTATCTTCA
601 -----+-----+-----+-----+-----+-----+-----+-----+ 660
CCTCTCCCTTACTAGAAGACTTCCACCTATACCATTACTATAATAGCAATAGAAAGT

a G E G N D L L K G G Y G N D I Y R Y L S -
GGATATGGCCATCATATTATTGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCT
661 -----+-----+-----+-----+-----+-----+-----+-----+ 720
CCTATACCGGTAGTATAACTGCTACTCCCCCTTCTGCTATTTGAGTCAAATCGA

a G Y G H H I I D D E G G K D D D K L S L A -
GATATAGATTTCCGGGACGTGCTTTAACGAGAAGGGAATGACCTCATTATGTATAAA
721 -----+-----+-----+-----+-----+-----+-----+-----+ 780
CTATATCTAAAGGCCCTGCAACGGAAATTGCTTCCCTTACTGGAGTAATACATATT

a D I D F R D V A F K R E G N D L I M Y K -
GCTGAAGGTAATGTTCTTCTATTGGCCACAAAATGGTATTACATTTAAAAACTGGTTT
781 -----+-----+-----+-----+-----+-----+-----+-----+ 840
CGACTTCCATTACAAGAAAGATAACCGGTGTTTACCATATGTAATTTGACCAAA

a A E G N V L S I G H K N G I T F K N W F -
GAAAAAGAGTCAGATGATCTCTCTAATCATCAGATAGAGCAGATTTGATAAAAGACGGC
841 -----+-----+-----+-----+-----+-----+-----+-----+ 900
CTTTTCTCAGTCTACTAGAGAGATTAGTAGTCTATCTCGTCTAAAACATTCTGCCG

a E K E S D D L S N H Q I E Q I F D K D G -
AGGGTAATCACACCAGATTCTCTTTAAAAAGCATTGAAATATCAGCAGAGTAATAACAAG
901 -----+-----+-----+-----+-----+-----+-----+-----+ 960
TCCCATTAGTGTGGTCTAAGAGAATTTCGTAACCTATAGTCGTCTCATTATTGTT

Fig. 8 (cont.)

a R V I T P D S L K K A F E Y Q Q S N N K -
 961 GTAAGTTATGTGTATGGACATGATGCATCAACTTATGGGAGGCCAGGACAATCTTAATCCA
 CATTCATAACACATAACCTGTAACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGT 1020

a V S Y V Y G H D A S T Y G S Q D N L N P -
 1021 TTAATTAAATGAAATCAGCAAAATCATTTCAGCTGCAGGTAACCTCGATGTTAAGGAGGA
 AATTAATTACTTTAGTCGTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCTT 1080

a L I N E I S K I I S A A G N F D V K E E -
 1081 AGATCTGCCGCTCTTATTGCAGTTGCCGGTAATGCCAGTGATTTTCATATGGACGG
 TCTAGACGGCGAAGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTACCTGCC 1140

a R S A A S L L Q L S G N A S D F S Y G R -
 1141 AACTCAATAACTTGACAGCATCAGCATAATATATTAAATTAAATTTAAATGATAGCAATCTTACT
 TTGAGTTATTGAAACTGTCGTAGTCGTATTATATAATTAAATTTACTATCGTTAGAATGA 1200

a N S I T L T A S A * -
 1201 GGGCTGTGCCACATAAAGATTGCTATTTGGAGTCATAATGGATTCTTGTCAATAAAT
 CCCGACACGGTGTATTCTAACGATAAAAAACCTCAGTATTACCTAACGAGACAGTATTAA 1260

1261 TGATTATGGTTATACGCCCTGGAGATTAGCCAATACCATAACGTCTCTGTTAACCC
 ACTAATACCCAAATATGCCGGACCTCTAAACGGTTATGGTATTGCAGAGACAATTGGG 1320

1321 GGAAGAAATTAAACATAGATTGACACAGACGGACTGGTCTGGGATTAACGTATGGTT
 CCTTCTTAATTGTATCTAAACTGTCGTGCCCTGACCAGACCTAACGGTACCAAA 1380

1381 GCTTGCTGCGAAATCTTAGAACTAAAGGTAAAACAGGTAAAAAAACAATTGACCGATT
 CGAACGACGCTTAGAAATCTGATTTCCATTGTCCATTGGTTACTGGCTAA 1440

1441 AAACTTTATTCCTCTGCCCGCATTAGTCTGGAGAGAGGATGGACGTCAATTATCTGAC
 TTTGAAATAAGAGACGGCGTAATCAGACCTCTCCTACCTGCAGTAAATAAGACTG 1500

Fig. 8 (cont.)

1501 TAAAGTCAGTAAAAGAAGCAAACAGATATCTTATTCTGATCTGGAGCAGCGAAATCCCCG
 1560 ATTCAGTCATTCTCGTTGTCTAGAATAAAGACTAGACCTCGTCGCTTAGGGGC

1561 TGTTCTCGAACAGTCTGAGTTGAGGCCTTATATCAGGGCATATTATTCTATCGCTTC
 1620 ACAAGAGCTTGTCAACTCAAACCGCAATATAGTCCCGTATAATAAGAATAGCGAAG

1621 CCGTTCTCTGTTGCCGGAAACTGGCGAAATTGACTTACCTGGTTATTCCGCCAT
 1680 GGCAAGAAGACAACGGCCCTTGACCGCTTAAACTGAAATGGACCAAATAAGGACGGTA

1681 TATAAAATACAGGAGAATATTATTGAAACCCCTGTTGTCTGTTTTTACAATTATT
 1740 ATATTATGTCCCTCTTATAAAACTTGGAACACACAGACAAAAATGTTAATAA

1741 TGCATTAATAACCCCCCTTTTTTCAGGTGGTTATGGACAAAGTATTAGTCACAGGGG
 1800 ACGTAATTATTGGGGGGAAAAAAAGTCACCAATACCTGTTCATATAACGTGTCCCC

1801 ATTTCAACTCTTAATGTTATTACTGTCGATTATCTGTTGTTGTTGAGATTAT
 1860 TAAAGTTGAGAATTACAATAATGACAGCGTAATAGACAACACCACAAACTCTAATA

1861 ACTCAGCGTTAAGAACTTACATTTGCACATAGTACAAGTCGGATTGATGTTGAGTT
 1920 TGAGTCGCAAATTCTGAATGTTAAACGTGTATCATGTTAGCCTAACTACAACCAA

1921 GGGGCCAAACTCTCCGGCATTTACTGGCGCTACCGATCTTATTTGAGAGTCGTCG
 1980 CCCACGGTTGAGAAGGCCGTAAATGACCGCGATGGCTAGAGAATAAAACTCTCAGCAGC

1981 TGTTGGTGTACTGTTGCCAGGGTAAGAGAATTAGACCAAGATCCGTAATTTCTGACAGG
 2040 ACAACCACTATGACAACGGTCCCATTCTTAATCTGGTAGGCATTAAAAGACTGTCC

2041 ACAGGCATTAACATCTGTTGGACTTATTATTCATTCAATTGGTAGGCATTAAAAGACTGTG
 2100 TGTCCGTAATTGAGACAAGACCTGAATAATAAAAGTAAGTATAAAAACGCCATTACAC

Fig. 8 (cont.)

HindIII

2101 GTATTACAGTCAAAGCTTACTCTGGTGATCTTATTTCGCTGCCTGTTATGCTGCATG
CATAATGTCAAGTTTCGAATGAGACCACTAGAATAAAAGCGACGGAACAATACGACGTAC 2160

2161 GTCTGTTTATTAGCCCCATTTGCGACGTCGCCTGATGATAAGTTCACCGGAATGC
CAGACAAAATAATCGGGTAAACGCTGCAGCGGAACACTATTCAAAAGTGCCTTACG 2220

2221 GGATAATCAATCTTCCTGGTGGAACATCAGTCACGGGATTAACACTATAAAAGCTATGGC
CCTATTAGTTAGAAAGGACCACCTAGTCAGTGCCGCTAATTGTGATATTCGATACCG 2280

PstI

2281 AGTCTCACCTCAGATGACGAACATATGGGACAAACAATTGGCAGGATATGTTGCTGCAGG
TCAGAGTGGAGTCACTGCTGTATACCTGTTGTTAACCGTCTATACAACCGACGTCC 2340

2341 CTTCAAAGTGACAGTATTAGCAACCATTGGCAACAAGGAATACAGTTAATACAAAAGAC
GAAGTTTCACTGTCATAATCGTTGGTAACCAAGTTGTTCTTATGTCATTATGTTTCTG 2400

2401 TGTTATGATCATCAACCTGTTGGGGAGCACACCTGGTTATTCGGGGATTTAAGTAT
ACAATACTAGTAGTTGGACACCAACCCCTCGTGTGGACCAATAAGGCCCTAAATTCTA 2460

2461 TGGTCAGTTAATTGCTTTAATATGCTTGGTCAGATTGTTGCAACCGGTTATCGCCT
ACCAGTCATTAACGAAAATTATACGAACGACCAGTCTAACACGTGGCCAATAAGCGGA 2520

2521 TGCACAAATCTGGCAGGATTCAGCAGGGTGGTATATCAGTTACCGCCTGGTGATGT
ACGTGTTAGACCGTCCTAACAGTCGTCAACCATAAGTCATGGCGGAACCACTACA 2580

2581 GCTTAACCTCCAAC TGAAAGTTATCATGGGAAACTGGCATTACCGGAAATTATGGTGA
CGAATTGAGAGGGTGA CTTCAATAGTACCCCTTGACCGTAATGGCCTTAATTACCACT 2640

2641 TATCACTTTCGTAATATCCGGTTTCGCTATAAGCCTGACTCTCCGGTTATTTAGATAA
ATAGTGAAGCATTATAGGCCAAGCGATATTGGACTGAGAGGCCAATAAAATCTATT 2700

2701 TATCAATCTCAGTATTAAGCAGGGGGAGGTATTGGTATTGTCGGACGTTCTGGTCAGG
ATAGTTAGAGTCATAATTGCCCCCTCCAATAACCATAACAGCCTGCAAGACCAAGTCC 2760

Fig. 8 (cont.)

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AAAAAGCACATTAACCTAAATTAAATTCAACGTT
2761 -----+-----+---+--- 2792
TTTTCGTGTATTGATTAAAGTTGCAA

Cutting enzymes:

BamHI HindIII NcoI PstI SalI SfiI

Not cutting enzymes:

EcoRI NotI

Fig. 8 (cont.)

PVamyZHLYA map

With 11 enzymes: ECORI SPHI PSTI NCOI NHEI NDEI BAMHI HINDIII
SALI SFII NOTI

1 ACTATAGGGAGACCACAAACGGTTCCCTCTAGAAATAATTTGTTAACCTTAAGAAGGA 60
 1 TGATATCCCTCTGGTGTGCCAAAGGGAGATCTTATTAAAACAATTGAAATTCTTCCT

NcoI
 61 GATATATCCATGGCTCAGGTGCAGCTGGTAGCTTGGGAGGCTCGGTGCAGGCTGGG 120
 61 CTATATAGGTACCGAGTCCACGTCGACCACCTCAGAACCCCTCCGAGCCACGTCCGACCC

a M A Q V Q L V E S W G G S V Q A G -
 |=> VHH amylase

121 GGGTCTCTGAGACTCTCCTGCACAGCCCCCTGGATTCACCTCCAATAGCTGCCCATGGAC 180
 121 CCCAGAGACTCTGAGAGGACGTGCGGGACCTAAGTGGAGGTTATCGACGGCTACCTG

a G S L R L S C T A P G F T S N S C R M D -

PstI
 181 TGGTACCGCCAGGCTGCAGGGAAGCAGCGAGTGGGTCTCATCTATTAGTACTGATGGT 240
 181 ACCAAGGCGGTCCGACGTCCCTCGTCGCGCTCACCCAGAGTAGATAATCATGACTACCA

a W Y R Q A A G K Q R E W V S S I S T D G -

241 CGCACAAAGCTATGCAGACTCCGTGAAGGGCGATTCAACCATCTCAAAGACAAAGCCAAG 300
 241 GCGTGTTCGATACGTCTGAGGCACCTCCCGCTAAGTGGTAGAGGTTCTGTTCGGTTC

a R T S Y A D S V K G R F T I S K D K A K -

301 GACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGCCATCTATTACTGT 360
 301 CTGTGCCACATAGACGTTACTTGTCGGACTTGGACTCTGTGCCGTAGATAATGACA

a D T V Y L Q M N S L K P E D T A I Y Y C -

361 GCCGTGAGGACGAATGGGTATCGTCCGCAATCTCACGAATTCGCTACTGGGGCCCGGGG 420
 361 CGGCACCTCGCTAACCCATAGCAGGCGTTAGAGTGCTAAAGCGATGACCCGGGCCCC

Fig. 9

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a A V R T N G Y R P Q S H E F R Y W G P G -

 SfiI SalI
 | |
 421 ACCCAGGTACCGTCTCCTCAACGGCCTGGGGCGCGTCACGTCGCCGGTCCGAAG
 TGGGTCCAGTGGCAGAGGAGTTGCCGGAGCCCCGGCGCAGCTGCAGGCCGCCAGGCTTC 480

 a T Q V T V S S T A S G A A S T S G G P K -
 |=> Ig hinge

 481 CCTTCCACTCCGCCGGTCTTCCGTATGAAACAGCTGGAAGACAAAGTAGA^gGAGCTC
 GGAAGGTGAGGCAGGGCCCAGAAGGGCATACTTGTCGACCTCTGTTCATCTTCGAG 540

 a P S T P P G S S R M K Q L E D K V E E L -
 |=> Leucine Zipper GCN4

 HindIII
 |
 541 CTTAGCAAGAACTACCACATCTAGAAAACGAGGTAGCTGCTGAAAAAGCTTGTGGTGA
 GAATCGTTCTGATGGTAGATCTTGCTCCATCGAGCAGACTTTTCGAACAACCACTT 600

 a L S K N Y H L E N E V A R L K K L V G E -

 SalI
 |
 601 CGTGGTGGTCACCATCACCATCACCATCGCTGACGCCGGGGTGCGCCGGTCCGTAT
 GCACCAACAGTGGTAGTGGTAGCTGCTGAGCTGCGGGCCCCACCGGGCACGGCATA 660

 a R G G H H H H H H A S T P G G A P V P Y -
 |=> 6xhis tag |=> E-tag

 BamHI
 |
 661 CCGGATCCGCTGGAACCGGCCGGgAAAATTCTCTTGCTAAAAATGTATTATCCGGTGG
 GGCTAGGCACCTGGCCGGCCcTTTAaGAGAACGATTTTACATAATAGGCCACCT 720

 a P D P L E P A G E N S L A K N V L S G G -
 |=> C-hlyA

 721 AAAGGTAATGACAAGTTGTACGGCAGTGAGGGAGCAGACCTGCTGATGGCCGGAGAAGGG
 TTCCCATTAAGTGTCAACATGCCGTACTCCCTCGTCTGGACGAACTACCGCCTTCCC 780

 a K G N D K L Y G S E G A D L L D G G E G -

Fig. 9 (cont.)

AATGATCTTCTGAAAGGTGGATATGGTAATGATATTATCGTTATCTTCAGGATATGGC
 781 -----+-----+-----+-----+-----+-----+-----+-----+ 840
 TTACTAGAAGACTTCCACCTATACCATTACTATAAATAGCAATAGAAAGTCCTATACCG

a N D L L K G G Y G N D I Y R Y L S G Y G -

CATCATATTATTGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCTGATATAGAT
 841 -----+-----+-----+-----+-----+-----+-----+-----+ 900
 GTAGTATAATAACTGCTACTTCCCCCTTCTGCTATTTGAGTCAAATCGACTATATCTA

a H H I I D D E G G K D D K L S L A D I D -

TTCCGGGACGTTGCCTTTAACCGAGAAGGAAATGACCTCATTATGTATAAAGCTGAAGGT
 901 -----+-----+-----+-----+-----+-----+-----+-----+ 960
 AAGGCCCTGCAACGAAATTCGCTTCCCTACTGGAGTAATACATATTCGACTTCCA

a F R D V A F K R E G N D L I M Y K A E G -

AATGTTCTTCTATTGCCACAAAATGGTATTACATTAAAAACTGGTTGAAAAAGAG
 961 -----+-----+-----+-----+-----+-----+-----+-----+ 1020
 TTACAAGAAAGATAACCGGTGTTTACCATATGAAATTGACCAAACCTTTCTC

a N V L S I G H K N G I T F K N W F E K E -

TCAGATGATCTCTAATCATCAGATAGAGCAGATTTTGATAAAAGACGGCAGGGTAATC
 1021 -----+-----+-----+-----+-----+-----+-----+-----+ 1080
 AGTCACTAGAGAGATTAGTAGTCTATCTCGTCTAAAACATATTCTGCCGTCCCATTAG

a S D D L S N H Q I E Q I F D K D G R V I -

ACACCGAGATTCTTAAAAAAGCATTTGAATATCAGCAGAGTAATAACAAGTAAGTTAT
 1081 -----+-----+-----+-----+-----+-----+-----+-----+ 1140
 TGTGGTCTAAGAGAATTTTCGTAACCTATAGTCGCTCTCATTATTGTTCCATTCAATA

a T P D S L K K A F E Y Q Q S N N K V S Y -

GTTATGGACATGATGCATCAACTTATGGGAGCCAGGACAATCTTAATCCATTAATTAAT
 1141 -----+-----+-----+-----+-----+-----+-----+-----+ 1200
 CACATACCTGTACTACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGTAATTAATTA

a V Y G H D A S T Y G S Q D N L N P L I N -

Fig. 9 (cont.)

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PstI

1201 GAAATCAGCAAAATCATTCAGCTGCAGGTAACCTCGATGTTAAGGAGGAAAGATCTGCC
CTTAGTCGTTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTTTCTAGACGG 1260

a E I S K I I S A A G N F D V K E E R S A -

NdeI

1261 GCTCTTTATTGCAGTTGTCGGTAATGCCAGTGATTTCATATGGACGGAACCTCAATA
CGAAGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTACCTGCCTTGAGTTAT 1320

a A S L L Q L S G N A S D F S Y G R N S I -

1321 ACTTTGACAGCATCAGCATAATATATTAATTAAATGATAGCAATCTTACTGGGCTGTGC
TGAAACTGTCGTAGTCGTATTATATAATTAAATTACTATCGTTAGAATGACCCGACACG 1380

a T L T A S A * -

1381 CACATAAGATTGCTATTTGGAGTCATAATGGATTCTGTCATAAAATTGATTATGG
GTGTATTCTAACGATAAAAAAACCTCAGTATTACCTAACAGTATTTAACTAACCTACC 1440

1441 GTTATACGCCCTGGAGATTTAGCCAATACCAACGTCTCTGTTAACCGGAAGAAAT
CAATATGCGGGACCTCTAAATCGGGTTATGGTATTGCAGAGACAATTGGCCTCTTA 1500

1501 TAAACATAGATTGACACAGACGGACTGGCTGGGATTAACGTACGGTGCTGCTGC
ATTTGTATCTAACTGTGTCGCCCCGACGACCTAACGTCAGTACCAACGAACGACG 1560

1561 GAAATCTTGAACTAAAGGTAAAACAGGTAAAAAAACAATTGACCGATTAAACTTAT
CTTAGAAATCTGATTTCCATTGTCCATTGGTAACTGGCTAACATTGAAATA 1620

1621 TTCTCTGCCGCATTAGTCTGGAGAGAGGATGGACGTCAATTCTGACTAAAGTCAG
AAGAGACGGGCGTAATCAGACCTCTCCTACCTGCAGTAAATAAGACTGATTTCAGTC 1680

1681 TAAAGAAGCAAACAGATATCTTATTCTGATCTGGAGCAGCGAAATCCCCGTGTTCTCGA
ATTTCTCGTTGTCTATAGAATAAGACTAGACCTCGTCGCTTAGGGGCACAAGAGCT 1740

Fig. 9 (cont.)

1741 ACAGTCTGAGTTGAGGCCGTATATCAGGGCATATTATTCTTATCGCTTCCCGTTCTTC
 1800 TGTCAGACTCAAACCTCCGAATATACTGCCCCGTATAATAAGAATAGCGAAGGGCAAGAAG

 1801 TGTTGCCGGAACTGGCGAAATTGACTTACCTGGTTTATTCTGCCATTATAAAAATA
 1860 ACAACGGCCCTTGACCGCTTAAACTGAAATGGACCAAATAAGGACGGTAATATTTAT

 1861 CAGGAGAATATTATTGAAACCCTGTTGTCTGTTTTTACAATTATTGCATTAAT
 1920 GTCCTCTTATAAATAACTTGGAACACAGACAAAAAAATGTTAATAAACGTAATTA

 1921 AACCCCCCTTTTTTCAGGTGGTTATGGACAAAGTATTAGTCACAGGGGATTTCAAC
 1980 TTGGGGGGAAAAAAAGTCCACCAATACCTGTTCATATACTCACGTGCCCCCTAAAGTTG

 1981 TCTTAATGTTTACTGTCGATTATCTGTTGTGGTGGTGGTGGAGATTATACTCAGCGG
 2040 AGAATTACAATAATGACAGCGTAATAGACAACACCACAACTCTAATATGAGTCGCC

 2041 TTTAAGAACTTACATTTGCACATAGTACAAGTCGGATTGATGTTGAGTTGGTGCAA
 2100 AAATTCTTGAATGTAACACGTGTATCATGTTCAGCCTAACTACAACCAACCGGTT

 2101 ACTCTTCCGGCATTACTGGCGCTACCGATCTCTTATTTGAGAGTCGTCGTGGTGA
 2160 TGAGAAGGCGTAAATGACCGCGATGGCTAGAGAATAAAACTCTCAGCAGCACACCACT

 2161 TACTGTTGCCAGGGTAAGAGAATTAGACCAAGATCCGTATTTCTGACAGGACAGGCATT
 2220 ATGACAACGGTCCCATTCTTAATCTGGCTAGGCATTAAGACTGTCCTGTCGTAA

 2221 AACATCTGTTCTGGACTTATTATTTCATTCATTTTGCCTAATGTTGAGAGTCGTCGTGGT
 2280 TTGTAGACAAGACCTGAATAATAAAAGTAAGTATAAAACGCCATTACACCATAATGTC

 HindIII
 |
 2281 TCCAAAGCTTACTCTGGTGTCTTATTTCGCTGCCTTGTATGCTGCATGGCTGTTT
 2340 AGGTTTCAATGAGACCACTAGAATAAAAGCGACGGAACAATACGACGTACCAAGACAAAA

 2341 TATTAGCCCCATTGCGACGTCGCCTTGTGATGATAAGTTTACGGAAATGCGGATAATCA
 2400 ATAATCGGGTAAACGCTGCAGCGGAACACTACTATTCAAAAGTGCCTTACGCCTATTAGT

Fig. 9 (cont.)

2401 ATCTTCCTGGTGGAAATCAGTCACGGCGATTAACACTATAAAAGCTATGGCAGTCTCAC
 2460 TAGAAAGGACCACCTTAGTCAGTGCCGCTAATTGTGATATTTCGATACCGTCAGAGTGG

NdeI	PstI
TCAGATGACGAACATATGGGACAAACAATTGGCAGGATATGTTGCTGCAGGCTCAAAGT	

2461 AGTCTACTGCTTGTATAACCCCTGTTAACCGTCCTATACAACGACGTCCGAAGTTCA

2521 GACAGTATTAGCAACCATTGGTCAACAAGGAATACAGTTAATACAAAAGACTGTTATGAT
 2580 CTGTCATAATCGTGGTAACCAGTTGTTCTTATGTCAATTATGTTCTGACAATACTA

2581 CATCAACCTGTGGTGGGAGCACACCTGGTTATTCGGGGATTTAAGTATTGGTCAGTT
 2640 GTAGTTGGACACCAACCCTCGTGTGGACCAATAAGGCCCTAAATTCTATAACCAGTCAA

2641 AATTGCTTTAATATGCTGGTCAGATTGTTGCACCGGTTATCGCCTTGACAAAT
 2700 TTAACGAAAATTATACGAACGACCAGTCAACACGTGGCAATAAGCGGAACGTGTTA

2701 CTGGCAGGATTCACCAGGGTGGTATATCAGTTACCCGCTTGGTGATGTGCTTAAC
 2760 GACCGTCCTAAAGGTGTCACCAACCATAAGTCAATGGCGGAACCACTACACGAATTGAG

2761 TCCAACGTAAAGTTATCATGGGAAACTGGCATTACCGGAAATTATGGTGATATCACTT
 2820 AGGTTGACTTTCAATAGTACCCCTTGACCGTAATGGCCTTAATTACCACTATAAGTGA
 2881 TCGTAATATCCGGTTTCGCTATAAGCCTGACTCTCCGGTTATTTAGATAATATCAATCT
 2880 AGCATTATAGGCCAAAGCGATATTCGGACTGAGAGGCCAATAAAATCTATTATAGTTAGA

2881 CAGTATTAAGCAGGGGGAGGTTATTGGTATTGTCGGACGTTCTGGTCAGGAAAAAGCAC
 2940 GTCATAATTGTCCCCCCTCCAATAACCATAACAGCCTGCAAGACCAAGTCCTTTCTG

2941 ATTAACATAATTCAACGTT
 2963 TAATTGATTAATTAAAGTTGCAA

Fig. 9 (cont.)

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Cutting enzymes:

BamHI HindIII NcoI NdeI PstI SalI SfiI

Not cutting enzymes:

EcoRI NheI NotI SphI

Fig. 9 (cont.)

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